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Testimony

Interactions of antibody-conjugated nanoparticles with biological surfaces

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Dedicated to Professor Ivan B. Ivanov (LCPE, University of Sofia) on the occasion of his 70th birthday.

I am delighted to write this article on the occasion of the 70th anniversary of Professor Ivan B. Ivanov and would like to thank Dr. Kralchevsky for the kind invitation. Dr. Kralchevsky and I wondered for a while whether my current work, mostly biological and even clinical, on the use of antibodies as therapeutics, would be of any interest to my former colleagues, colloid chemists. However, a few months ago the direction of research in our laboratory (the former Laboratory of Experimental and Computational Biology) veered toward nanotechnology with biological applications, or nanobiology. Nanotechnology, when applied to cancer research, can be broadly defined as anything between 1 and 100 nm that contains a man-made component and can help in the fight against cancer. Importantly, this range of sizes distinguishes based on size-related properties. Interestingly, I began my scientific explorations 34 years ago, under the tutelage of Dr. Ivanov, in the field of thin liquid films, which begin to exhibit their size-dependent thermodynamic properties precisely when their thickness falls below about 100 nm, behaving similarly are small droplets. I now have the opportunity to, in a way, return to my scientific roots and gear that experience toward biology and medicine. Perhaps, the knowledge accumulated for decades of research on thin films could find new applications in the exciting area of nanotechnology. In any case, even if a comparison between the study of thin liquid films and cancer-fighting agents may appear to lack sound scientific basis, I hope to, in the rest of this article, describe certain aspects of my current work and future plans that could potentially be of interest to colloid chemists. But first, I would like to recount the beginnings of my scientific career under Professor Ivanov's guidance, so as to provide a background for the subsequent technical discussion.

1. Doing science under Professor Ivanov's supervision: both enjoyable and arduous?

As a 17-year-old freshman at the Faculty of Chemistry in 1971, I first heard of the study of thin liquid films. Perplexed by the existence of films thinner than 100 nm, yet fascinated by the prospect of exploring such a topic, I approached Professor Ivanov, the recognized expert in theory of thin liquid films, about the possibility of working with him. I vividly remember our first meeting in his small office at the end of the corridor; I was so excited and proud that a well-known professor like himself was willing to discuss with me and even give me a project. My scientific career began with several conditions: I had to quickly learn English in order to expand my access to pertinent literature, and I had to read Levich's classic "Physicochemical Hydrodynamics", as well as several articles on thin liquid films. I viewed the reading as enjoyable, and a necessary prelude to doing the real science that I held sacred. Soon enough, I was using a method developed by Drs. B. Radoev and Ivanov in an attempt to account for the effect of surfactant surface diffusion on the rate of thinning of planar liquid films. I was surprised to find that the thinning rate was increasing, compared to the rate calculated by the Reynolds equation, as the thickness decreased. Professor Ivanov was delighted because the surface diffusion could affect the critical thickness of rupture suggesting a possible mechanism of its dependence on thickness, and also because my observations could offer a new method for measurement of the surface diffusion coefficient. I was exalted – I had found a new formula, and the emotionality that comes with discovery still stays with me. The possibility of this formula being important, and of contributing to Professor Ivanov's research, was certainly one of the most enjoyable moments in my life as a scientist. At the time, I did not understand too well why Professor Ivanov insisted on going through the arduous process of checking and rechecking calculations, and when everything seemed

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so clear at that! However, I resigned my skepticism, checked and rechecked, and continued on in Professor Ivanov's laboratory. And today, I require the same painstaking meticulousness and precision from my postdocs; hopefully, the pleasure that lies behind simply doing science more than compensates in their point of view, as it did for me in my first interactions with science.

2. Nanoparticles and colloid particles – are they the same?

Solid particles, liquid droplets and gas bubbles, and the liquid films between them in colloid systems have been known for decades to possess size-dependent properties that differ from those of the same material in the bulk when any size is smaller than about 100 nm. Recent advances in physics, chemistry, materials sciences, engineering, and molecular biology, have allowed the development of nanoparticles (size 1–100 nm) by combining atoms or molecules one at a time, and in arrangements that do not occur in nature. Such particles have attracted much attention because of their unique mechanical, electrical and optical properties. This resulted in a renewed interest to various nanoparticles already known for many years, e.g., liposomes, and to the development of new ones, e.g., quantum dots and gold nano-shells. Such particles conjugated to antibodies can improve their binding or/and effector functions or confer new functions, e.g., cytotoxicity, size-dependent fluorescence and light scattering. Compared to antibody engineered as fusion proteins or conjugated chemically to other compounds, the nanoparticle-antibody conjugates have the fundamental capability of separating compounds loaded inside the particle, e.g., liposomes, from the outside environment. In addition, because of the relatively high volume to surface ratio of the nanoparticle, the concentration of loaded active substances, e.g., imaging agents, can be much higher than for antibody fusion proteins or complexes. It is also relatively easy to design multifunctional nanoparticle-antibody conjugates that combine targeting, imaging and therapeutic properties. Finally, the nano size of these particles still allows penetration of different organs, and at the same time can confer relatively long half-life in the circulation of animals. Thus, nanoparticle-antibody conjugates can compliment existing diagnostic tools and treatment protocols and offer entirely new possibility for diagnosis and treatment of diseases.

For the purposes of diagnosing and treating cancer, any particle of size in the range from 1 to 100 nm can be called nanoparticle but with an important addition – it must be multifunctional and also should contain a man-made component, thus biological molecules of this size or their assemblies alone are excluded from this definition. For example, with this definition cell organelles or viruses are not nanoparticles even if their size is in the nanorange. However, a liposome (a vesicle from a bilayer lipid membrane) in the nanosize range is a nanoparticle, and to distinguish from other liposomes, e.g., cell-size liposomes, we call it now a nanoliposome. If this nanoliposome contains drug enclosed inside its membrane, and at the surface has directing molecules, example, antibodies, then it becomes a multifunctional nanoparticle because it has two functions: (1) encapsulates drug that can increase its half-life and prevent from degradation, and deliver to certain organs, and (2) can specifically bind to molecules of choice, in particular, cell surface receptors, that can ensure directed delivery of the drug to cells of importance for cancer. An ideal multifunctional nanoparticle for use against cancer would also have a signaling component so it can diagnose cancer and assess the therapeutic effect. In addition, it could have a triggering property so it can be made to release the drug only after reaching the target.

How is this related to colloid chemistry? Indeed there are ample opportunities for colloid chemists to contribute to nanobiology (for our purposes nanobiology is the science behind the development of nanosize particles containing biomolecules but assembled in a way that does not exist in biological systems). I briefly discussed above nanoliposomes as an example of a potentially useful nanoparticle system. However, liposomes have been around for relatively long time since Bangham discovered them more than 30 years ago. Although there are some success stories with liposomes used for medical purposes, the high expectations years ago have not been met. Thus, new-targeted delivery nano systems are urgently needed. They can be based on nanodroplets, nanobubbles and solid nanoparticles interacting with biological surfaces.

3. Interactions of antibody-conjugated nanoparticles with biological surfaces – how important are they for the elimination of death and suffering due to cancer?

The major purpose to develop nanoparticles for biomedical purposes in most cases is for targeting cells. Thus, understanding the mechanisms of the interaction of nanoparticles with biological surfaces is important for at least two major reasons: (1) by increasing specificity targeting can be made much more efficient, and (2) decreasing non-specific binding not only may help to increase efficiency but even more importantly could decrease possible toxic effects.

As we know from colloid chemistry, interaction of particles with surfaces at close apposition depends primarily on two major factors: (1) thermodynamic that can be estimated by the free energy of interaction and (2) kinetic that depends on the viscosity and geometry. I have been working under Dr. Ivanov's supervision on the second factor and years ago I summarized our results and results of others that may have some biological relevance in a review [1] published 22 years ago which I still find useful. As far as I know conceptually things have not changed so much since. If the separation between the two surfaces is on average much smaller than any other dimension then certain approximations known as the thin film approximations can be applied which reduce the complex Navier–Stokes equations and allow in some cases analytical solutions. As the separation decreases the viscous friction increases and the rate of approach begins to decrease compared to that at infinite separation and the same driving force. Such an approach could be applied even for describing the kinetics of approach of large biological molecules to surfaces. In particular, we are currently modeling the interaction of antibodies with charged nanoparticles by using the Poisson–Boltzmann equation solved at constant

charge or constant surface potential. The rate of approach of these (and other) large molecules can then be calculated by using thin film approximations. Similarly the rate of antibody-conjugated nanoparticles approaching cells can be estimated by knowing the interaction forces and using the thin film approximation.

After attachment an antibody-conjugated nanoliposome can either stay at the cell surface or be endocytozed and delivered to endosomes. In both cases the fate of the liposome would depend whether it can fuse spontaneously with the plasma or the endosomal membrane, and if it can how quickly it will before it is trafficked to lysosomes where it can be degraded. Thus, even if targeted the efficacy of delivery of any compounds encapsulated inside liposomes would not be so high because generally membranes do not fuse spontaneously – they are designed by nature to be stable and separate cells or intracellular compartments from outside environment. Therefore, currently we are trying to design nanoliposomes that can fuse with membranes by incorporating membrane fusion machineries borrowed from viruses. Indeed viruses appear perfect vehicles for targeted delivery and in many aspects their interactions with biological surfaces resembles interactions of nanoliposome-antibody conjugates. For example, enveloped viruses enter cells by interaction of their envelope glycoproteins (Env) with cell surface receptors, then fusing their membranes with the cell membranes and delivering their genome inside cells [2]. However, the Env, which is the targeting component of the viruses, is difficult to be retargeted without affecting the ability of the viral fusion machinery to fuse membranes. In addition, it is difficult to encapsulate significant amounts of additional molecules inside viruses. How to combine the universal targeting ability of antibodies conjugated to nanoliposomes, which can encapsulate any chosen compound, with the ability of the viral Env to fuse membranes?

There are at least two approaches to do this – simple and sophisticated. The simple approach is straightforward – incorporate into the liposomal membrane any nonspecific viral fusion machinery (nonspecific meaning an Env that can fuse membranes provided they are at close apposition but does not require any specific Env-receptor interaction that can trigger conformational changes required for fusion). To confer specificity of targeting, an antibody should be also incorporated into the liposomal membrane. Currently, Dr. Robert Blumenthal and I, and our associates, are developing this approach for specific delivery of drugs into cancer cells. Although nanoliposomes developed by this approach presumably would deliver toxic agents to cancer cells more efficiently than nanoliposomes without Envs, these liposomes could also fuse with any cells to which they can bind nonspecifically causing toxicity. In the ideal case the nanoliposomes should fuse with only those cells that express the specific tumor antigens they are targeted too. The sophisticated approach therefore requires that the antibody binding domain is incorporated into a fusogenic protein (e.g., viral Env or cell protein) in such a way that its binding to the tumor antigen should trigger conformational changes leading to fusion at the plasma cell membrane or endocytosis with subsequent fusion at low pH. (Most fusogenic proteins fall into two classes in dependence of how the conformational changes leading to fusion are triggered – receptor-induced or low pH-triggered.) We and others have been trying to develop such protein-based fusion machineries incorporated into liposomes for highly efficient targeted delivery of drug, genes, markers and other compounds into any cell of choice [3,4]. Although there are no major breakthroughs the current focus on nanotechnology has revived the interest to this fundamental challenging problem and hopefully we can solve it in the near future.

Suppose we have such wonderful nanoliposomes which can interact with any cancer cell of choice, then whenever instructed or after interaction with the tumor antigen they fuse with the cell membrane, be the plasma one or endosomal, and delivery their cargo to kill, modify or make visible the cell of choice. Is this going to cure cancer? Or other diseases? Obviously it is difficult to answer by yes or no such a question. Cancer, e.g., is not one disease but there are hundreds of different cancers, and even the same type of cancer can vary from individual to individual. A fundamental problem for the application of liposomes in the diagnosis and treatment of cancer is related to difficulties in penetration of solid tumors where even large molecules as antibodies cannot get easily inside. For some cancers, e.g., leukemias, where the cancer cells are mostly in the blood and easy to reach, liposomes could be very efficient. However, for such cancers antibodies alone also show efficiency in some cases, although not in very significant proportion of patients. What is the advantage of the targeted nanoliposomes in this case? There are several, and some were already mentioned above; the most important one is that targeted fusing nanoliposomes can deliver drugs highly efficiently and in the ideal case without any toxic effects. Therefore we believe that these vehicles could lead to elimination of all cancer cells they can reach. In the case of easily accessible cancer cells, as leukemic cells, they can be used at high effective concentrations and destroy all cancer cells thus leading to cure.

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